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Journal of Photochemistry and Photobiology B: Biology 83 (2006) 94–97
Journal of Photochemistry and Photobiology B:Biology
www.elsevier.com/locate/jphotobiol

The effect of skin permeation enhancers on the formation of porphyrins in mouse skin during topical application of the methyl ester of 5-aminolevulinic acid

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Received 20 July 2005; received in revised form 23 November 2005; accepted 9 December 2005
Available online 26 January 2006

Abstract

The influence of skin permeation enhancers, such as dimethyl sulphoxide (DMSO) and 1-[2-(decylthio)ethyl]azacyclopentan-2-one (HPE-101), Labrafac®CC, Labrafil®, Labrasol® and Transcutol® in a concentration of 10% (wt./wt.) on the formation of porphyrins in normal mouse skin from topical application of creams with methyl 5-aminolevulinate (MAL) was studied. The concentration of porphyrins in the mouse skin was determined by direct fluorescence measurements. The results show that studied permeation enhancers increase the formation of porphyrins, and therefore also the skin penetration 2% MAL whereas for 10% and 20% (wt./wt.) MAL concentrations only DMSO, HPE-101 and Labrafac®CC increased the porphyrin formation. At all studied MAL concentrations DMSO gave the largest enhancing effect, similarly to that of HPE-101. This suggests that in 2–20% MAL creams HPE-101 may be substituted by Labrafac®CC to reduce skin irritation induced by HPE-101 without impairing the porphyrin formation.
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Keywords: Porphyrins; Normal mouse skin fluorescence; In vivo direct skin fluorescence measurement; Methyl 5-aminolevulinate; Topical application; Permeation enhancers

1. Introduction

The 5-aminolevulinic acid (ALA), the precursor of porphyrin biosynthesis, is applied for fluorescence diagnosis and photodynamic therapy (PDT) of skin cancers [1–3]. The use of ALA has several advantages over traditional PDT, where photosensitizing dyes are injected intravenously or given orally. Firstly, ALA can be applied topically because it is small enough to penetrate the stratum corneum. Secondly, ALA-induced porphyrins are eliminated more rapidly from the organism than other sensitizers, and therefore gives shorter lasting cutaneous photosensitization [1–3].

A limitation of ALA-PDT is the shallow penetration of ALA into tissues. Thus, ALA esters, which are more lipophilic than ALA, were introduced to PDT to get a deeper penetration and facilitate the treatment of larger tumors [4,5]. In some cases so-called penetration enhancers are added to ALA and its esters formulations [6–10].

In the present work, the influence of selected skin permeation enhancers on porphyrin formation from ALA esters was studied. The concentration of porphyrin formed is supposedly a measure of methyl ALA ester (5-aminolevulinate, MAL) penetration. Direct in vivo fluorescence measurements were used to determine the porphyrin concentration in the upper tissue layers.

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doi:10.1016/j.jphotobiol.2005.12.003

2. Materials and methods

2.1. Chemicals

MAL was purchased from Sigma Chemical Co. (St. Louis, MO). HPE-101 was a gift from Hisamitsu Pharmaceuticals (Tokyo, Japan). Labrafac®CC, Labrafil®, Labrasol® and Transcutol® were kindly supplied from Gattefossé (Saint Priest, France). All other reagents and solvents were of analytical grade and were used as purchased.

2.2. Animals

Inbred female albino nude mice (BALB/c, Norwegian Radium Hospital, Oslo, Norway) were used. The mice experiments were performed according to the European Convention for the Protection of Vertebrates Used for Scientific Purposes at the Norwegian Radium Hospital (Oslo, Norway) with ethical approval from the National Animal Research Authority.

2.3. Fluorescence measurement

A fiber-optic probe was coupled to a Perkin-Elmer LS50B luminescence spectrometer (Norwalk, CT) for in vivo fluorescence measurements. The probe was made of a commercially available fiber accessory (Perkin-Elmer, two 1 m fused silica fiber bundles joined in parallel at the measuring tip) fitted with a cylinder shaped aluminum spacer. This spacer was 6.5 mm in diameter and provided a constant, fixed distance of 10 mm between the fibers and the tissue. This assured a relatively uniform light distribution over the measuring area and provided the maximum fluorescence signal for a given sample. Fluorescence intensity was measured at the application site as a function of time. The excitation wavelength was set at 407 nm and fluorescence was measured at 636 nm. The 407 nm light from the spectrometer was of low intensity (1.5 $\mu W/cm^2$) and did not induce photobleaching.

2.4. Application of MAL

Prior to application of MAL, the animals were anesthesized intraperitonneally with Equithesin® (Rikshospitalet, Oslo, Norway) or by inhalation of Fluothane (Cyphrane Ltd. Keighley, Yorkshire, United Kingdom). Creams containing MAL were prepared in the standard ointment (Unguentum Merck, Germany). Approximately 0.1 g of the cream was applied to one flank of the mice. The application site (1 cm of diameter) was covered with an adhesive dressing (opSite Flexigrid, Smith and Nephew Medical Ltd., Hull, England) during the whole application period. Application of the cream was performed under different conditions for different groups, three animals per data point.

2.5. Continous application

MAL cream was applied with concentration 2%, 10% or 20% (wt./wt.). Fluorescence measurements were performed up to 24 h. The fluorescence could be measured through the transparent occlusion tape, because the absorbance by the tape was negligible.

2.6. Effect of skin permeation enhancers

In the control group, creams containing 2%, 10% or 20% (wt./wt.) MAL without enhancer was applied. To six groups, were treated with creams containing 2%, 10% or 20% (wt./wt.) MAL supplemented with 10% (wt./wt.) of DMSO, HPE-101, Labrafac®CC, Labrafil®, Labrasol® or Transcutol® were applied. The fluorescence was measured as described above [6].

3. Results and discussion

After application of creams containing 10% of DMSO, HPE-101 or Labrafac®CC an increase of porphyrin fluorescence was observed for all studied MAL concentrations, suggesting that these permeation enhancers increase bioavailability of MAL to the mouse skin. As shown in the Fig. 1a, the maximum fluorescence of 2% MAL in the presence of DMSO or HPE-101 was reached at about 6 h, and its value was about 1.7-times higher than that in the control sample.

When MAL was applied at a concentration of 10% (wt./wt.), the porphyrin formation was the best in the presence of 10% (wt./wt.) DMSO. In this case the maximum of fluorescence was observed at about 5 h. As for 2% MAL it was about 1.7-times higher than that of the control sample. For HPE-101 and Labrafac®CC these maxima were respectively 1.5- and 1.3-fold higher that of the control (Fig. 1b). Similarly, in the case of 20% (wt./wt.) MAL cream, the enhancers in concentration 10% increased the porphyrin formation in a similar manner as for the creams with 10% MAL (Fig. 1c). For all studied enhancers the fluorescence maxima were reached at about 5 h.

Surprisingly, in the case of creams supplemented with 10% of Labrafil®, Labrasol® or Transcutol® an increase of porphyrin formation, compared to that in the control sample without enhancers, was observed only for 2% MAL (Fig. 1a). For higher concentrations of MAL Labrafil® gave no significant effect on porphyrin formation while Labrasol® and Transcutol® inhibited the formation significantly (1b and c). These phenomena are difficult to explain. Especially, the difference between effect of Labrafac® CC and other enhancers is a challenging question. The reason for this difference may be differences in the mechanism of action. Labrafac®CC (a mixture of medium-chain triglycerides) probably acts as a stratum corneum emollient [11–16], similarly to DMSO [9,10,12] and HPE-101 [6,12,17,18], while Labrafil®, Labrasol® and

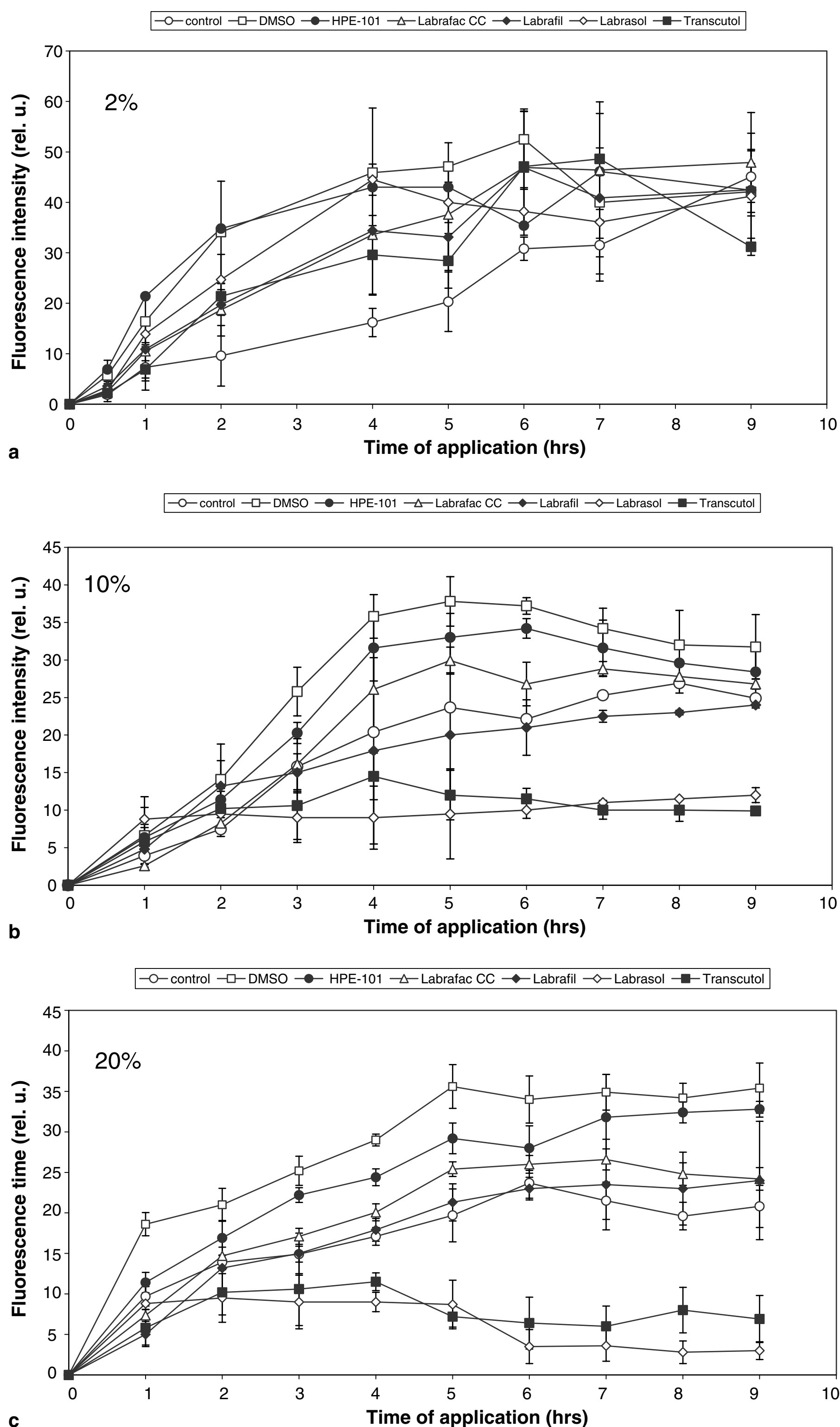

Fig. 1. In vivo porphyrin fluorescence kinetics in nude mouse skin after topical application of MAL 2% (a) 10% (b) 20% (c) in creams containing 10% of skin permeation enhancers. The control sample was MAL creams without any permeation enhancers. The data prints show the mean from three measurements. Error bars indicate the standard deviation.

Transcutol® (containing ethylene glycol ethers and esters) act rather as emulsifiers and solubilizers of MAL [11,13,19,20], so that their activity would be limited by a ratio of drug and cream basis concentrations [11,20]. On the other hand, the effects of these substances observed for 10% and 20% MAL did not change with increasing concentration (Fig. 1b and c). Thus, no firm conclusion concerning mechanisms can be drawn.

In conclusion, our results demonstrate significant effects of penetration enhancers on the time-dependent kinetics of MAL induced porphyrins in tissues. The porphyrin production in the skin after application of 2%, 10% and 20% (wt./wt.) MAL creams do not depend on the concentration of MAL suggesting that the heme pathway of porphyrin metabolism is saturated [6]. Ten percent (wt./wt.) DMSO, HPE-101 and Labrafac®CC increased the penetration of MAL into intact mouse skin. The similarity of the effects of Labrafac®CC and HPE-101 suggests that by Labrafac®CC would be a good alternative for HPE-101 in the MAL creams, since it may give less skin irritation than HPE-101. A surprising and yet not understood effect was the decrease of porphyrin formation observed after application of Labrasol® and Transcutol®.

4. Abbreviations

ALA 5-aminolevulinic acid
DMSO dimethyl sulfoxide
HPE-101 1-[2-(decylthio)ethyl]azacyclopentan-2-one
MAL methyl 5-aminolevulinate
PDT photodynamic therapy
wt./wt. weight/weight concentration

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